

Occurrence of the entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin in different tillage regimes and in *Zea mays* L. and virulence towards *Ostrinia nubilalis* (Hübner)

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ABSTRACT

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The entomopathogen, *Beauveria bassiana* (Balsamo) Vuillemin, is an important component in the corn (*Zea mays* L.) agroecosystem; this pathogen was found in soil, living corn plants, and field corn residue. *Beauveria bassiana* occurs naturally in the soil within different tillage systems (plow, chisel, no-tillage), with an average of 51–74 colony forming units g⁻¹ of soil. The natural inoculum was present in the crop residue from the different tillage systems, killing up to 84% of European corn borer larvae (*Ostrinia nubilalis* (Hübner), Lepidoptera: Pyralidae) overwintering in the no-tillage regime. Of the larvae collected from corn late in the season, 100% of those killed by *Beauveria bassiana* were from plants colonized by *Beauveria bassiana* applied foliarly at the whorl-stage ($\chi^2 = 19.1$, $P < 0.01$). However, the lack of mycosis in the total *O. nubilalis* population may have been a function of low concentrations of the fungus in the plants. Fungal samples isolated from internodal and nodal tissue of those plants maintained virulence, killing from 23–100% of exposed larvae. *Beauveria bassiana* has potential for environmentally safe insect suppression, in that it occurs naturally and can also be applied to field corn. Understanding the unique relationship between the soil, *Beauveria bassiana*, and *Z. mays* will be invaluable in furthering development and utilization of such fungi to manage insect pests of food plants.

INTRODUCTION

A novel endophytic relationship between an entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, and corn plants provides the basis

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for a new environmentally compatible insect suppressant. The European corn borer, *Ostrinia nubilalis* (Hübner), a primary pest of corn, *Zea mays* L., causes significant physiological damage to corn by feeding on leaves and ears and by tunneling into the stalk. Currently, this insect is managed in the United States by using host-plant resistance and rescue treatments with chemical insecticides, which do not always provide adequate control. The bacterium, *Bacillus thuringiensis* Berliner, is an efficacious alternative to chemical insecticides for *O. nubilalis* suppression. Resistance to *Bacillus thuringiensis*, however, has been documented in insects other than *O. nubilalis* (McGaughey, 1985; Goldman et al., 1986; Stone et al., 1989). In addition, there is much ongoing research with *Bacillus thuringiensis* to develop transgenic plants and microbes. By using transgenic plants, the likelihood of selecting for biotypes of *O. nubilalis* resistant to this microbial insecticide is increased.

The novel endophytic relationship between the entomopathogenic fungus, *Beauveria bassiana*, and corn plants is proposed as a potentially simpler and less expensive approach than gene transfer to obtain an environmentally sound alternative to chemicals for control of *O. nubilalis*. *Beauveria bassiana* has been used experimentally to suppress *O. nubilalis* populations for more than 60 years (Bartlett and Lefebvre, 1934; Stirrett et al., 1937; Beall et al., 1939; York, 1958; Riba, 1984; Lewis and Cossentine, 1986; Marcandier and Riba, 1986; Feng et al., 1988, Lewis and Bing, 1991). Most research has focused on the suppression of the insect by the fungus, but has not investigated the relationship between *Beauveria bassiana* and corn.

Many fungi are known to colonize plants, usually as plant parasites or symbionts. Endophytic fungi that do show toxicity towards insects may produce feeding deterrents and/or antibiotics (Clay et al., 1985a,b; Hardy et al., 1985), but are not insect pathogens as defined by Koch's postulates. *Beauveria bassiana* is different because (i) it is an entomopathogen that colonizes a green plant and (ii) it has not been documented as a plant pathogen. In addition, there is no evidence of *Beauveria bassiana* co-evolving with the corn plant (Pirozynski and Hawksworth, 1988) or interacting specifically with the plant (Kosuge and Nester, 1987). Therefore, the endophytic relationship between this entomopathogenic fungus and the corn plant is unique compared with other plant/fungal relationships.

Description of this phenomenon coincides with the international commitment to research directed towards alternative agricultural practices. Development of such alternatives is imperative. Concern over pollution of groundwater by chemical pesticides is increasing (Hallberg, 1987; Williams et al., 1988). Recently, a number of pesticides have been removed from use in the United States because of environmental concerns, and a disturbed public is demanding stricter controls.

In a study investigating the response of inbred corn lines to mycopathogenic fungi, *Beauveria bassiana* was fortuitously isolated from the pith of

plants for the first time (Vakili, 1990). The potential for suppression of *O. nubilalis* by *Beauveria bassiana* persisting on the surface of the corn plant has been suggested (Lewis and Cossentine, 1986). Season-long suppression of this insect pest has been correlated with the presence of *Beauveria bassiana* in corn pith at senescence (Lewis and Bing, 1991).

This fungal-plant relationship was examined experimentally by applying *Beauveria bassiana* to whorl-stage corn plants by foliar application of a granular formulation of conidia and by injection of a conidial suspension (Bing and Lewis, 1991). By harvest, 98.3% of the foliarly treated plants and 95.0% of the injected plants were colonized by *Beauveria bassiana*. *Beauveria bassiana* was isolated from 33.3% of the untreated plants, indicating colonization by naturally occurring inoculum. *Beauveria bassiana* applied by foliar application or injection significantly reduced tunneling by *O. nubilalis* compared with tunneling in the untreated plants. The authors concluded that *Beauveria bassiana* can colonize the corn plant at whorl stage, move within the plant, and persist to provide season-long suppression. It is not known how *Beauveria bassiana* enters the corn plant. Movement within the plant is possibly passive within the vascular system of the plant.

In an additional study, Bing and Lewis (1992a) demonstrated that *Beauveria bassiana* applied foliarly at whorl stage acts as a crop protectant by killing *O. nubilalis* larvae on whorl-stage corn. However, *Beauveria bassiana* did not persist to provide suppression of larvae on late-whorl or pretassel-stage corn. As the season progressed, a greater area of the plants was colonized by *Beauveria bassiana* as the fungus moved throughout the plants.

In a third study (Bing and Lewis, 1992b), *Beauveria bassiana* injected into plants at anthesis colonized the plants, moved primarily upward, possibly with plant photosynthates in the vascular tissue, and significantly reduced *O. nubilalis* tunneling. The colonization of corn plants was independent of *O. nubilalis* infestation; that is, the larvae were not transferring the fungus into the plants.

In this paper, data are presented from experiments designed to demonstrate: (i) the natural inoculum of *Beauveria bassiana* in the soil in different tillage regimes; (ii) the virulence of *Beauveria bassiana* in the natural population; (iii) the relationship between the presence of *Beauveria bassiana* in the plant and development of *Beauveria bassiana* infection in larvae collected from those plants; (iv) the virulence of the applied *Beauveria bassiana* after isolation from corn plants.

MATERIALS AND METHODS

Prevalence of Beauveria bassiana in soil

In this study, soil samples (20 per tillage system per sample date) were randomly collected over a three year period (Table 1) to determine the prev-

TABLE 1

Colony forming units (CFU) of *Beauveria bassiana* per gram of soil for samples collected from no-tillage, plow, and chisel-plow tillage systems in 1988–1990

Year	No-tillage (CFU g ⁻¹)	Plow (CFU g ⁻¹)	Chisel (CFU g ⁻¹)	Sample dates
1988	162	45	80	13 April, 4 May, 23 May, 14 November
1989	51	45	47	27 March, 10 May, 7 June, 7 July, 8 December
1990	10	37	25	9 April, 14 May
Mean	74	42	51	

absence of *Beauveria bassiana* occurring naturally in three different tillage systems: no tillage, plow, and chisel plow. The rotation was corn–following–corn and was initiated in 1982, replacing a corn–soybean rotation. On each sample date, soil was collected using a sterilized metal cylinder 7.0 cm wide \times 4.5 cm high. Soil samples were weighed, dried at 27°C for 2 days, serially diluted, and plated on selective agar (Doberski and Tribe, 1980). The number of colony forming units (CFU) g⁻¹ of soil was determined after incubation at 26°C for 10 days.

Virulence of Beauveria bassiana in natural populations

The virulence of *Beauveria bassiana* in the natural population was determined by observing *O. nubilalis* larvae that had overwintered in crop residue. These observations were made in the tillage systems from which the previous soil samples were collected. One-hundred cornstalks or substantial pieces of stalk were randomly selected from each of the no-tillage, plow, and chisel-plow systems on 3 June, 1991. The no-tillage system was also sampled on 11, 14, and 17 June; however, the plow and chisel-plow systems could not be sampled on these dates because the fields had been tilled for planting. The corn stalks were cut open, and the number of *O. nubilalis* larvae present and the number of cadavers exhibiting a mycosis from *Beauveria bassiana* were recorded. Pupal cases from which European corn borer adults had emerged were recorded as an uninfected insect form. Presence or absence of *Beauveria bassiana* in the overwintering population was analyzed using the χ^2 test.

Relationship between endophytic Beauveria bassiana and mycosis in larvae

Larvae for this evaluation were collected throughout the growing season from plants that had the fungus applied to the foliage or injected into the plant in an experiment designed to demonstrate the temporal relationships between the fungus, the corn plant, and *O. nubilalis* (Bing and Lewis, 1992a). The original *Beauveria bassiana* applied to the corn was isolated from the soil at the Iowa State University Research Farm, Ankeny (ARSEF 3113, USDA-ARS Entomopathogenic Fungi Collection, Ithaca, NY). Larvae collected from these plants were placed on laboratory diet (Guthrie et al., 1971), and incubated at 27°C, 70% r.h. Larvae were observed until mycosis developed or until they pupated. Five plants from each treatment (control, foliar, injected) in each of four replications were sampled on 21 June 1989, 2 July 1989 and 19 July 1989. A total of 180 plants were split and 1623 *O. nubilalis* larvae were collected. The presence of *Beauveria bassiana* in the corn plants and the development of *Beauveria bassiana* infection in larvae collected from those plants was analyzed using the χ^2 test.

Virulence of endophytic Beauveria bassiana

The endophytic *Beauveria bassiana* used to test for virulency came from corn tissue (internodal and nodal) samples which were taken using sterile techniques from plants in the study described in the previous section (Bing and Lewis, 1992a). Virulence (in this text virulent means the isolate killed some of the exposed larvae) was verified by covering early fifth-instar *O. nubilalis* larvae with the inoculum (Bing and Lewis, 1992b), placing the larvae on laboratory diet (Guthrie et al., 1971), and incubating at 27°C, 70% r.h., until conidiophores developed or the insects pupated. Isolates of *Beauveria bassiana* collected from plants during the growing season (before anthesis) and isolates collected from the plants late in the season (after anthesis) were both tested. For each of these two collection periods (before and after anthesis), 30 larvae per treatment (control, foliar, and injected) were used with four replications. A total of 120 larvae per treatment were tested for each collection period (720 larvae in total).

RESULTS AND DISCUSSION

Prevalence of Beauveria bassiana in soil

The number of CFU g⁻¹ of soil was highly variable between tillage systems and between years (Table 1). In 1988, the no-tillage system had the greatest number of CFU g⁻¹ of soil, as initially expected because the no-tillage system does not disturb the soil as do plow and chisel practices, but this trend was

not as evident in 1989 and 1990. Even within a given year and tillage practice the CFU g⁻¹ of soil varied greatly depending on sample date (e.g., from 200 g⁻¹ on 27 March 1989 to 0 g⁻¹ on 10 May 1989 in the no-tillage system). Therefore, although *Beauveria bassiana* does occur naturally in different tillage systems, the amount of inoculum varies greatly and probably is influenced more by environmental conditions than by tillage practices.

Virulence of Beauveria bassiana in natural populations

The incidence of *Beauveria bassiana* in the overwintering larvae in corn residue in the no-tillage, plow, and chisel-plow systems was examined (Table 2). In all three systems, the naturally occurring *Beauveria bassiana* reduced the number of *O. nubilalis* larvae by 31–61%. The percentage of larvae with *Beauveria bassiana* in the crop residue from the no-tillage and plow systems was twice that in the chisel plow system. This difference is significant, $\chi^2 = 7.0$, $P < 0.01$. Interestingly, the CFU g⁻¹ of soil in the chisel plow was not much different from that in the other two systems (Table 1). Even greater mortality was exhibited in samples taken later in the spring from the no-tillage system. On 11 June, 1991, 83% of the 64 overwintering larvae collected had been killed by *Beauveria bassiana*. Likewise, 84% of 49 larvae and 82% of 56 larvae collected on 14 and 17 June, respectively, had been killed by *Beauveria bassiana*. Obviously, naturally occurring *Beauveria bassiana* was suppressing the natural overwintering population of *O. nubilalis* in June 1991 for all three tillage systems. In another corn ecosystem, more than 60% of the diapausing *O. nubilalis* larvae collected from six geographical regions in France by Marcandier and Riba (1986) died from mycosis caused by *Beauveria bassiana* within 60 days of field collection. A strong enzootic of the fungus was observed between 1978 and 1983, in which time mortality levels of field-collected samples increased from 6% in 1978 to 90–98% in 1983. Because *Beauveria bassiana* occurs as a saprophyte in soil (McCoy et al., 1988), different agronomic methods should be investigated to ensure conservation of this natural inoculum.

TABLE 2

Incidence of *Beauveria bassiana* in *Ostrinia nubilalis* larvae collected 3 June 1991 that overwintered in different tillage systems

	No-tillage	Plow	Chisel
<i>O. nubilalis</i> per 100 plants	36	36	26
<i>O. nubilalis</i> with <i>B. bassiana</i>	61*	61*	31*

*significantly different at $P < 0.01$, $\chi^2 = 7.1$.

Relationship between endophytic Beauveria bassiana and mycosis in larvae

Of the larvae collected from corn late in the season (5 July and 19 July, 1989), 100% of those killed by *Beauveria bassiana* were from plants colonized by *Beauveria bassiana* (Table 3; $\chi^2=19.1$, $P<0.01$). However, of the total 1007 *O. nubilalis* collected, most (994) were not infected by the fungus, regardless of the presence or absence of the fungus in the plants. When the data for *O. nubilalis* collected on all dates (21 June, 5 July and 19 July, 1989) was combined, 40 of the 67 (60%) *O. nubilalis* killed by *Beauveria bassiana* were collected from plants colonized by the fungus (Table 4; $\chi^2=4.9$, $P<0.01$). However, of the total 1623 *O. nubilalis* collected, most (1556) were not infected by the fungus, regardless of the presence or absence of the fungus in the plants. Although most of the infected larvae came from plants colonized by *Beauveria bassiana*, not all larvae from colonized plants were infected. The lack of mycosis in the total *O. nubilalis* population may have been a function of low concentrations of the fungus in the plants. If this phenomenon is to be used in a field situation for suppression of *O. nubilalis*, it would be desirable to develop methods to increase the concentration of the fungus in the plant. Also, perhaps greater mycosis would have been exhibited if a

TABLE 3

Mycosis in *Ostrinia nubilalis* collected late in the season (5 July and 19 July 1989) from corn plants colonized and not colonized by *Beauveria bassiana*

	Corn plants with no <i>B. bassiana</i>	Corn plants with <i>B. bassiana</i>	Total
<i>O. nubilalis</i> with <i>B. bassiana</i> ¹	0	13	13
<i>O. nubilalis</i> without <i>B. bassiana</i> ¹	596	398	994
Total	596	411	1007

¹ $\chi^2=19.1$, $P\leq 0.01$.

TABLE 4

Mycosis in *Ostrinia nubilalis* collected throughout the season (21 June, 5 July and 19 July 1989) from corn plants colonized and not colonized by *Beauveria bassiana*

	Corn plants with no <i>B. bassiana</i>	Corn plants with <i>B. bassiana</i>	Total
<i>O. nubilalis</i> with <i>B. bassiana</i> ¹	27	40	67
<i>O. nubilalis</i> without <i>B. bassiana</i> ¹	841	715	1556
Total	868	755	1623

¹ $\chi^2=4.9$, $P\leq 0.01$.

fourth sample of larvae had been collected from these plants after the larvae had overwintered in the corn stalks. This would have given the larvae more time to be exposed to the *Beauveria bassiana* in the plants.

Virulence of endophytic Beauveria bassiana

In 1988, 79% of *Beauveria bassiana* isolated from plants during the growing season (before anthesis) were virulent to *O. nubilalis* larvae, whereas in 1989, 95% of the isolates were virulent. Only 23% of late-season samples after anthesis) in 1988 were virulent, whereas after anthesis in 1989, 100% of the *Beauveria bassiana* isolates were virulent to the *O. nubilalis*. Physiological changes in the plant during maturation had no negative impact on the virulence of the fungus. The more extreme environmental conditions (above-average temperatures and below-average rainfall) in 1988 may have contributed to the differences in virulence between years. Because the *Beauveria bassiana* isolated from the colonized plants was virulent, colonized plants may be another source of inoculum in a field situation for suppression of *O. nubilalis*.

CONCLUSIONS

Beauveria bassiana is indigenous to the corn agroecosystem; the fungus occurs naturally in different tillage systems and suppresses natural populations of *O. nubilalis* in the different corn systems. Therefore, enhancement of this pathogen by modifying agronomic practices is promising as a suppression technique. Isolates of *Beauveria bassiana* applied to whorl-stage corn foliarly or by injection remain virulent to *O. nubilalis*. Therefore, further investigation of ways to use this phenomenon to suppress *O. nubilalis* is warranted. Additional research on this endophytic relationship is needed to obtain a better understanding of the mode of entry, persistence, and morphological state of the fungus inside the plant. Once these are known this relationship can be more readily exploited for management of *O. nubilalis*.

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